

COMPARATIVE ANATOMY OF MATURE *THEMEDA TRIANDRA* FORSK. LEAF BLADES: A CORRELATED LIGHT AND ELECTRON MICROSCOPE STUDY

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ABSTRACT

Aspects of the anatomy of mature leaf blades of *Themeda triandra* Forsk. from the Fish River, Alice and Hogsback provenances were investigated with light and electron microscopes. The blades contain three orders of vascular bundle, each of which is surrounded by a bundle sheath consisting of relatively thick-walled, chlorenchymatous cells with centrifugally arranged, agranal chloroplasts. The bundle-sheath walls are variably lignified; in addition, the outer tangential and radial walls contain a continuous suberin lamella, which is widest at the sites of the plasmodesmatal aggregates. The phloem in all three vein orders has both thick- and thin-walled sieve tubes similar to those recorded in the longitudinal bundles of other grasses. In median and large (first-order) bundles, the phloem is bordered laterally by a layer of thick-walled cells. The laminae of specimens from Hogsback and Alice contained a greater percentage volume of air space (6,68 % and 6,06 %, respectively) than that from Fish River (3,78 %).

UITTREKSEL

DIE VERGELYKENDE ANATOMIE VAN VOLWASSE *THEMEDA TRIANDRA* FORSK. BLARE: 'N GEKORRELEERDE LIG- EN ELEKTRONMIKROSKOOP STUDIE

Die anatomie van volwasse *Themeda triandra* blare, afkomstig uit die Visrivier-, Alice- en Hogsback-gebiede, is deur middel van lig- en elektronmikroskopie bestudeer. Die drie herkenbare aarordes is in die blare, omring deur 'n eenlagige bondelskede wat uit taamlike dikwandige selle bestaan. Teen die buitenste tangensiale en radiale bondelskede wande kom korrellose chloroplaste voor. Die bondelskede se buitenste tangensiale en radiale wande besit 'n suberien lamella, wat verdik is waar die plasmodesmata, wat in primêre stippel velde gerangskik is, deurdring. Die phloem van al drie aarordes besit dik en dun wandige phloem – altesaam dieselfde as wat in ander grassoorte tevore beskryf is. Die grootaar en ook die van die primêre aarorde van die blaas, is omring deur 'n laag dikwandige selle. Groter inter- en primêre lugruimtes is ontwikkel in blare afkomstig van Hogsback (6,68 %) en Alice (6,06 %), as wat die geval is met *T. triandra* van die Visriviergebied (3,78 %).

1. INTRODUCTION

Themeda triandra Forsk. – four varieties of which are recognised by Chippindall in Meredith (1955) – forms part of the climax succession stage in savanna areas of Southern Africa. In the Eastern Cape, *T. triandra* var. *imberbis* occurs in at least three, differing veld types under widely differing climate regimes: from the low-lying, hot, semi-arid Fish River Valley area to the relatively cool, moist highland Hogsback area, with the Alice area intermediate between the two extremes (Acocks, 1975). The foliage leaves of plants from Fish River and Alice provenances are generally more glabrous and narrow than those of plants from the Hogsback provenance.

The anatomy of *Themeda* has been described by Goosens and Theron (1934) at the light microscope level, based on freehand sections and drawings made from these sections. It was felt that the illustrations in Goosens and Theron (1934) contain inaccuracies which precluded the use of much of the descriptive leaf anatomy of *Themeda* in these authors' paper.

The present study was undertaken to determine whether any anatomical differences existed in the leaves of plants from the three provenances – differences that might be attributed to the widely differing conditions under which this C_4 grass grows.

2. MATERIAL AND METHODS

T. triandra var. *imberbis* (Retz.) A. Camus tillers from Fish River, Alice and Hogsback provenances were collected from experimental plots established by Downing and Marshall (1980) in 1979. Tillers were potted and transferred to conviron EF 7-H environmental cabinets where they were grown under the following climatic regimes: *Themeda* (Fish River) 30°C max., 20°C min.; 16h photoperiod; RH 65 % max., 50 % min. *Themeda* (Alice) 28°C max., 18°C min.; 16h photoperiod; RH 70 % max., 50 % min. *Themeda* (Hogsback) 25°C max., 12°C min.; 16h photoperiod, RH 80 % max., 60 % min. Voucher specimens are lodged at both the University of Fort Hare and the National Herbarium, Pretoria, South Africa (Botha¹, Fish River; Botha², Alice; Botha³, Hogsback).

2.1. Fixation and Embedment

Tissues from the mid third of fully-expanded, mature laminae were selected and cut into small pieces approximately 3 × 5 mm. Samples were fixed on either 3 % or 6 % glutaraldehyde in buffer (0.05 or 0.025 M phosphate or cacodylate at pH 7.2). Cold fixation was carried out for 24 h, with frequent changes of the buffered fixative. The material was then trimmed into smaller pieces (approximately 2 × 2 mm), rinsed in the appropriate buffer and fixed in 2 % osmium tetroxide, which was made up in the

appropriate buffer solutions. Dehydration was in an alcohol series, followed by two changes in propylene oxide. Embedment was in Spurr's (1969) low viscosity resin.

2.2. Light and Electron Microscopy

Serial transverse, longitudinal, and paradermal sections, 0.5 to 2.0 μm thick, were cut with glass knives, on an LKB Ultratome III. Such sections were routinely stained in 0.005 % aqueous toluidine blue-O, or aqueous methylene blue, pH 6.8 followed by 0.05 % neo-fuchsin, on a hotplate for 30 seconds to 1 minute (all Merck chemicals).

Serial transverse 15 μm -thick sections were cut on a Leitz freezing microtome and stained with Sudan IV, for the presence of suberin, and ruthenium red for pectic material. Similar sections were treated with Phloroglucinol-HCl for the presence of lignins (Johansen, 1940). Results were recorded on film with integral 35 mm and 90×120 mm plate cameras on a Zeiss Photomicroscope III fitted with brightfield, phase and Nomarski interference contrast objectives.

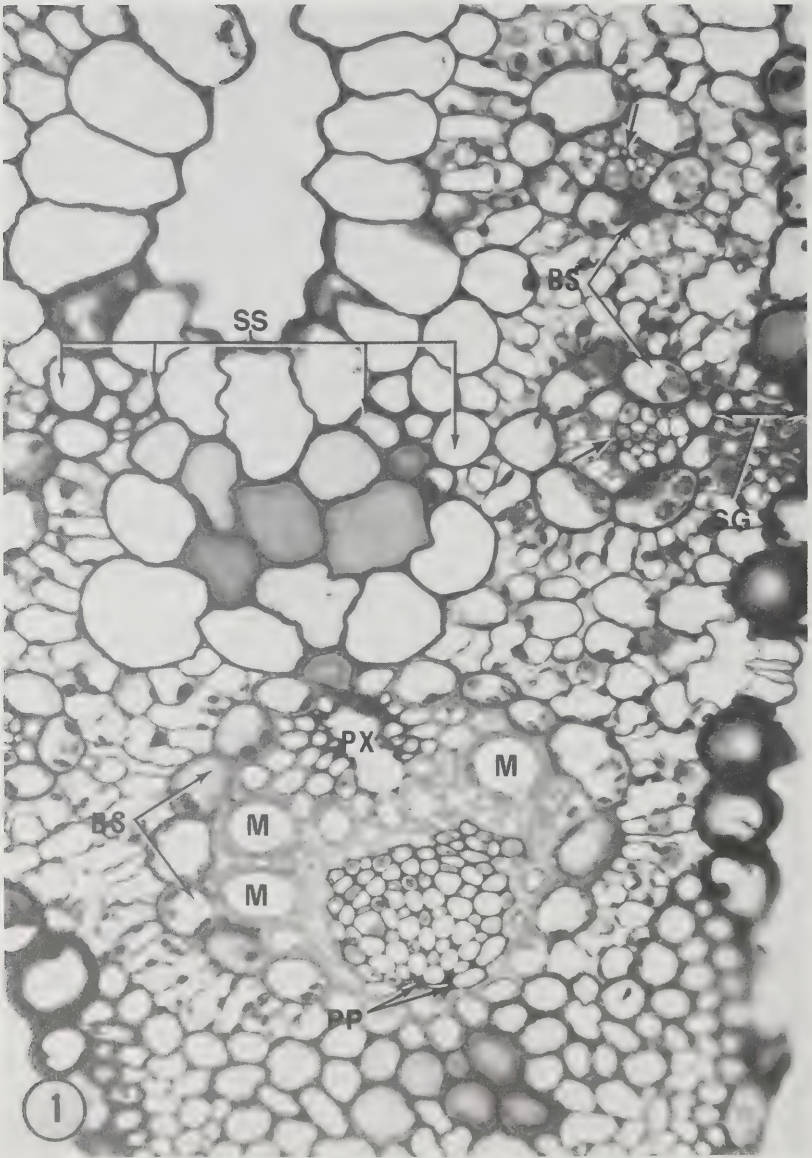
Ultra-thin serial sections were cut with diamond knives (DuPont), stained in uranyl acetate and lead citrate and viewed with an Hitachi HU-IIB electron microscope.

2.3. Leaf Air Space Volume

Segments $10 \text{ mm} \times 4 \text{ mm}$ were excised from the mid portion of fully expanded laminae. Ten similar-sized leaves were used for each of the above collections, the combined segments were weighed on a four decimal place Sartorius balance. The leaf material was immersed in a beaker containing 1 % Extran (Merck chemicals), to help break the surface tension, placed in a dessicator, and subjected to a mild vacuum (-500 mbar) for 4 min. The vacuum was released rapidly, and re-applied for a further three infiltrations, after which the segments were removed, blotted dry of surface water and quickly weighed. Segments were considered to be surface-dry if no sheen was visible on leaf surfaces. Experiments were replicated four times for each provenance. The technique used here is in essence, similar to that previously reported by Byott (1976).

3. RESULTS

For the sake of brevity, the three provenances of *Themeda triandra* reported on herein will be referred to a *Themeda* Fish River (TFR), *Themeda* Alice (TA), and *Themeda* Hogsback (TH). Where no distinctions are made, descriptions apply equally to all three provenances.



3.1. Brief Description of the Leaf Blade

The mature leaf blades of *T. triandra* are narrow and tapering. As viewed in transverse section, they are either shallow or steeply V-shaped, and exhibit the Kranz structure typical of C_4 grasses: a concentric radial layering of mesophyll and chloroplast-rich bundle-sheath cells around the vascular bundles. The vascular bundles together with their associated Kranz layers (the bundle sheath and Kranz mesophyll) are separated from one another by narrow zones of non-Kranz mesophyll cells, or so-called 'translucent cells' (Ellis, 1976).

In addition to a median bundle, three orders, or classes, of vascular bundles can be recognized:

- (a) First order, or large, bundles (Figs 2, 4). These bundles are characterised by the presence of large metaxylem vessels on either side of the protoxylem, which often is represented by a lacuna. Obliterated protophloem is evident on the abaxial surface. Conspicuous sclerenchyma girders extend from the bundles to both upper and lower epidermis.
- (b) Second order, or intermediate, bundles (Figs 1, 2, 6). These bundles lack large metaxylem vessels and protoxylem lacunae. Hypodermal sclerenchyma strands or girders occur on both ad- and abaxial surfaces, or on the abaxial surface only.
- (c) Third order, or small, bundles (Figs 1, 2, 4, 6, 8). In addition to lacking large metaxylem vessels and protoxylem lacunae, these bundles are not associated with either hypodermal sclerenchyma strands or girders.

3.2 Detailed Description of the Vascular Bundles

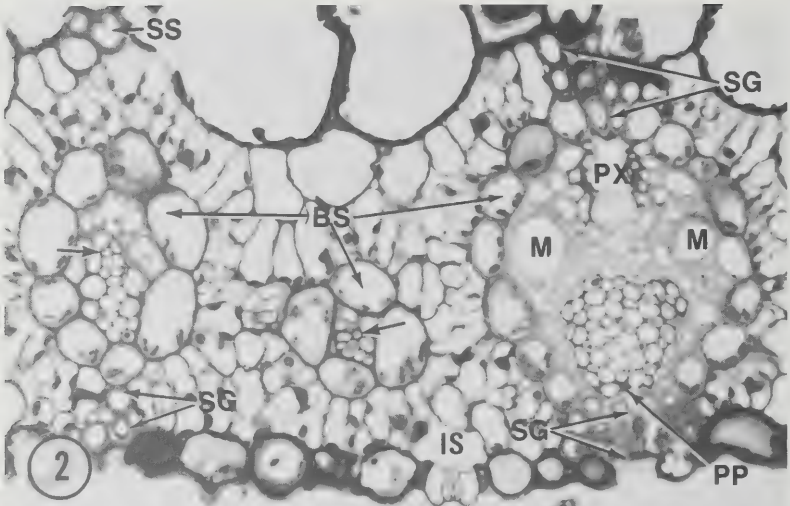
3.2.1 Median Bundle

The midrib contains a single median bundle bordered on both sides by chlorenchymatous bundle-sheath cells, which are in direct contact with Kranz mesophyll cells. On both upper and lower sides of the bundle, the sheath cells are virtually achlorophyllous. The ground tissue above the bundle consists of large parenchyma cells and below of collenchymatous cells. The cells of the upper epidermis are bulliform cells.

The median bundle in Figure 1 (TFR) is representative of the median bundles in all three provenances. Like the large bundles, median bundles contain large metaxylem vessels on either side of a protoxylem lacuna. In

FIG. 1.

Transection of part of mature *Themeda* (Fish River) leaf blade, showing (bottom to top) median, intermediate, and small vascular bundles. BS = chlorenchymatous bundle-sheath cells; M = metaxylem vessel; PP = obliterated protophloem; PX = protoxylem lacuna; SG = sclerenchyma girder; SS = sclerenchyma strand; unlabelled arrows point to thick-walled sieve tubes. Toluidine blue. X 660.



the median bundle of Figure 1 two large vessels are found on the left and only one on the right. As many as four large vessels may be present, with two on both sides of the lacuna. The large metaxylem vessels are always in direct contact with the chlorenchymatous bundle sheath cells. The protoxylem lacuna is bordered laterally by nonlignified parenchyma cells and abaxially by a row of metaxylem elements that extend toward the large metaxylem vessels on either side.

The median bundle contains both protophloem and metaphloem. By the time the median bundle is mature, the protophloem, which occurs on the lower surface, is obliterated. The remaining metaxylem consists of sieve-tube members and parenchymatous elements, including companion cells. Laterally, the phloem is separated from the chlorenchymatous bundle sheath by a layer of thick-walled cells.

3.2.2. Large Bundles

Figures 2 and 4 provide transverse views of large bundles from Fish River and Alice provenances, respectively. The large bundles of *T. Hogsback* are similar to these.

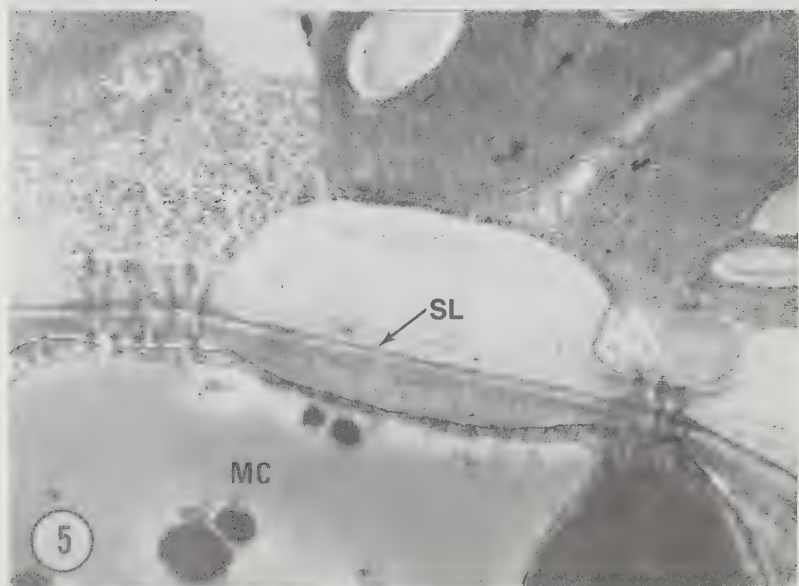
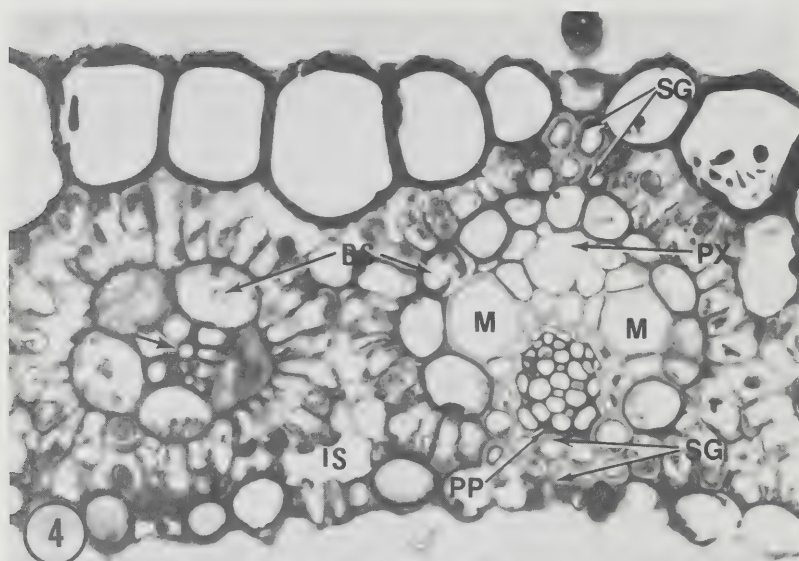
As mentioned previously, the large bundles contain large metaxylem vessels and a protoxylem lacuna. No more than two such vessels—one on either side of the lacuna—have been encountered in any large bundle. The protoxylem lacuna in the large bundle of Figure 4 is smaller, and less conspicuous than that in the large bundle of Figure 2. Similar variation may be encountered among bundles of the same leaf section. A secondary wall thickening of an intact protoxylem element can be seen beneath

FIG. 2.

Transection of part of mature *Themeda* (Fish River) leaf blade, showing (left to right) intermediate, small, and large vascular bundles. Both intermediate and small bundles are entirely surrounded by a chlorenchymatous bundle sheath (BS); that of the large bundle is disrupted ad- and abaxially by sclerenchyma girders (SG). IS = intercellular space; M = metaxylem vessel; PP = obliterated protophloem; PX = protoxylem lacuna; SS = sclerenchyma strand; unlabelled arrows point to thick-walled sieve tubes. Toluidine blue. X 560.

FIG. 3.

Electron micrograph showing a chlorenchymatous bundle-sheath cell, which was associated with a small vascular bundle in a leaf blade of *Themeda* (Fish River). The agranal chloroplasts contain numerous starch grains. A suberin lamella (SL) can be seen in the outer tangential and radial walls of this cell. The arrowheads point to aggregates of plasmodesmata in walls between bundle-sheath cells (above, left), and between the bundle-sheath cell and a mesophyll cell (right, with widened suberin lamella). IS = intercellular space; MC = mesophyll cell; N = nucleus of bundle-sheath cell. 3% glutaraldehyde, 2% OsO₄ in 0.025 M phosphate buffer. X 10500.



the lacuna in Figure 2. Nonlignified parenchyma cells may or may not border the lacuna. Relatively small metaxylem elements bridge the gap between large metaxylem vessels, which are in direct contact with the chlorophyllous bundle sheath.

The large bundles contain both protophloem and metaphloem. As in the median bundle, the protophloem is obliterated. Although not always apparent with the light microscope, the electron microscope has revealed that the metaphloem contains two kinds of sieve tubes, thin-walled ones and thick-walled ones. The thick-walled sieve tubes are among the last to form and occur just outside the xylem. Both phloem parenchyma cells and companion cells are found in large bundles.

All large bundles are associated with sclerenchyma girders, some of which are merely in contact with the chlorenchymatous bundle sheath; others actually disrupt it. In Figure 2, the chlorenchymatous sheath of the large bundle is disrupted on both ad- and abaxial surfaces, whilst that of the large bundle in Figure 4 is disrupted on the abaxial surface only. Notice the layer of thick-walled cells bordering the phloem immediately inside the chlorenchymatous bundle sheath.

3.2.3. Intermediate Bundles

Unlike median and large bundles, intermediate bundles lack protoxylem lacunae and large metaxylem vessels typically associated spatially with such lacunae. Although the xylem consists entirely of metaxylem, both protophloem and metaphloem may be present.

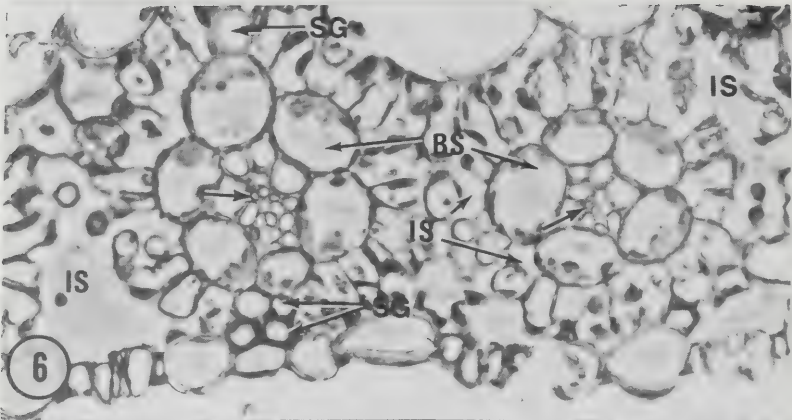
The phloem consists of sieve-tube members, companion cells, and parenchyma cells. Distinct xylem parenchyma cells apparently are lacking in most intermediate bundles. Parenchyma cells in direct contact with tracheary elements generally are also in direct contact with sieve tubes. Moreover, the

FIG. 4.

Transection of part of mature *Themeda* (Alice) leaf blade, showing small (left) and large (right) vascular bundles. The chlorenchymatous bundle sheath (BS) of the large bundle is disrupted only abaxially by a sclerenchyma girder (SG). IS = intercellular space; M = metaxylem vessel; PP = obliterated protophloem; PX = protoxylem lacuna; unlabelled arrow points to thick-walled sieve tube in small bundle. Toluidine blue. X 680.

FIG. 5.

Electron micrograph showing aggregates of plasmodesmata in mesophyll bundle-sheath cell wall of *Themeda* (Alice) leaf blade. Note that the suberin lamellae (SL) is wider at the sites of plasmodesmata. Portions of agranal chloroplasts can be seen in the bundle-sheath cell (above). MC = mesophyll cell. 6% glutaraldehyde, 2% OsO₄ in 0.05 M phosphate buffer. X 21 600.



contents of these highly vacuolate cells differ little, if any, from those of the parenchyma cells of the phloem. For these reasons, all of the parenchymatous cells of intermediate bundles, with the notable exception of companion cells, will be referred to as "vascular parenchyma cells." All intermediate bundles apparently contain both thin- and thick-walled sieve tubes. The thick-walled sieve tubes often are in direct contact with tracheary elements.

As mentioned previously, intermediate bundles are associated with hypodermal sclerenchyma strands or girders on both ad- or abaxial surfaces, or on abaxial surfaces only. Figures 1, 2 and 6 serve especially to illustrate this aspect. Most of the intermediate bundles are completely enclosed by a chlorenchymatous bundle sheath. The bundle sheath of the intermediate bundle in Figure 1, however, is almost disrupted on its abaxial surface by a hypodermal sclerenchyma girder. On its adaxial surface, an extension of the bundle sheath is in contact with a hypodermal sclerenchyma strand. In Figure 2, the chlorenchymatous bundle sheath is almost completely encircled by Kranz mesophyll cells, as the hypodermal sclerenchyma strand, above, lacks contact with the bundle sheath; below is a small girder.

Finally, in Figure 6, small girders occur both above and below the intermediate bundle.

3.2.4. Small Bundles

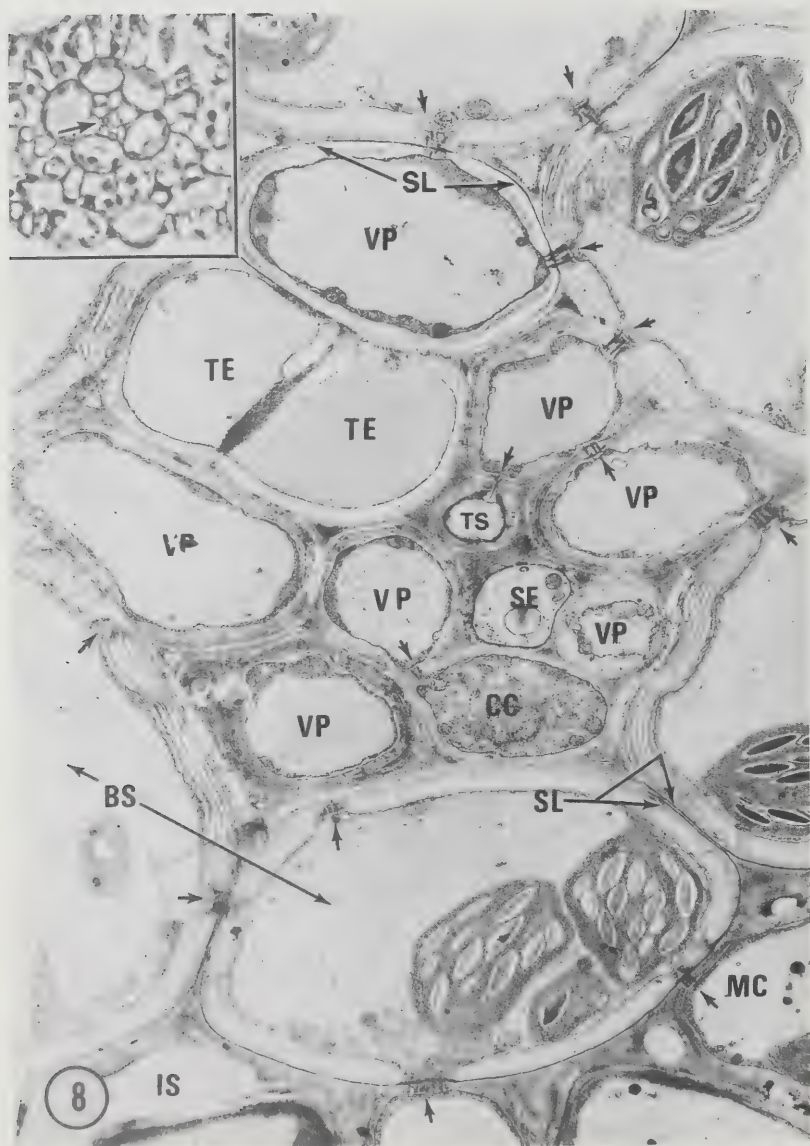
It is the small bundles, consisting entirely of metaxylem and metaphloem, that are completely encircled by chlorenchymatous bundle sheaths and mesophyll cells that radiate out from the sheaths. Transverse views of small bundles from the three provenances are illustrated in Figures 1 and 2 (TFR), 4 (TA), and 6 and 8 (TH); and in longitudinal view in Figures 9–11 (TFR, TA, and TH, respectively).

FIG. 6.

Phase-contrast micrograph of part of mature leaf blade of *Themeda* (Hogsback), showing intermediate (left) and small (right) vascular bundles. Both bundles are surrounded by a chlorenchymatous bundle sheath (BS), and both contain thick-walled sieve tubes (unlabelled arrows) next to the xylem. The intermediate bundle is associated with sclerenchyma girders (SG) both ad- and abaxially. IS = intercellular space. X 700.

FIG. 7.

Electron micrograph showing portions of bundle-sheath cells (BS) and adjacent mesophyll cell (MC) in *Themeda* (Hogsback). The agranal chloroplasts of the bundle-sheath cells contain numerous starch grains. Arrowheads point to plasmodesmata in walls between bundle-sheath cells and between a bundle-sheath cell and mesophyll cell. IS = intercellular space. 6% glutaraldehyde, 2% OsO₄ in 0.05 M cacodylate buffer. X 8650.



As with intermediate bundles, except for the companion cells, all of the parenchymatous cells of the small bundles are referred to as vascular parenchyma cells. All small bundles apparently contain at least one thick-walled sieve tube and one thin-walled sieve tube. Commonly, the thick-walled sieve tubes are in direct contact with tracheary elements.

3.3. The Bundle-sheath Cells

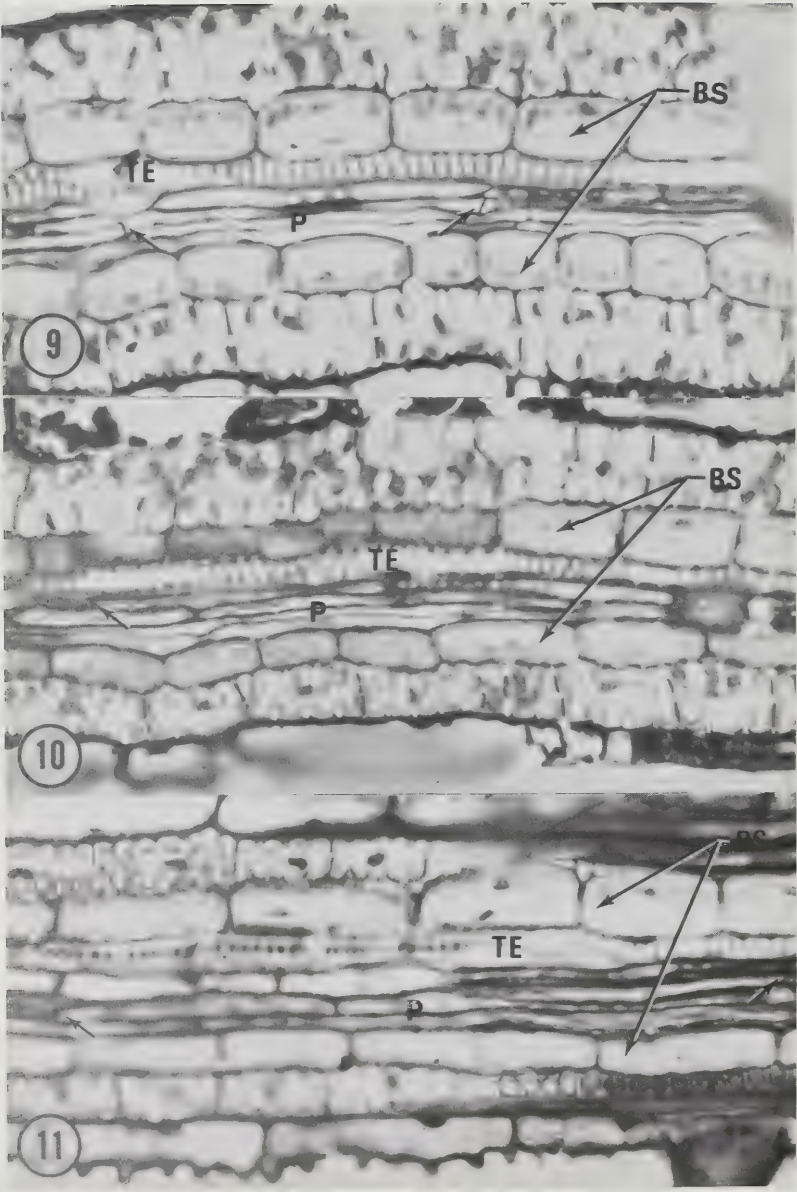
The walls of the bundle-sheath cells in all provenances are appreciably thicker than those of contiguous mesophyll cells (Figs 3, 5, and 7). In addition, all of the bundle-sheath cell walls have a more or less lamellate appearance, with those of TH being most conspicuously lamellate (Figs 7 and 8) and those of TFR being least so (Fig. 3). The lamellate appearance in TH material post-fixed in osmium tetroxide is so conspicuous that it can be used as a reliable criterion at the electron-microscope level to distinguish TH from TFR or TA material. Examination of both fresh, free-hand sections and thinner, freeze-microtomed sections treated with phloroglucinol-HCl, indicate that the electron-opaque portions of the walls correspond to more heavily lignified regions.

When viewed in transverse sections, the outer tangential and radial walls of all bundle-sheath cells exhibit a continuous suberin lamella. The suberin lamella is conspicuously wider in the regions of the wall between bundle-sheath cells or bundle-sheath cells and mesophyll cells transversed by aggregates of plasmodesmata (Figs 3, 5, 7, 8). The plasmodesmata are aggregated in the pit-membranes of the bundle-sheath cell walls. Two suberin lamellae occur in the radial walls between adjacent sheath cells, one on either side of the compound middle lamella (Figs 3 and 8).

Suberin lamellae also occur in the outer tangential and radial walls of cells that fill the gaps in the chlorenchymatous bundle sheaths resulting from the presence of sclerenchyma girders. In addition, the outer tangential and radial walls of vascular parenchyma cells bordering tracheary elements may be suberised (Fig. 8).

FIG. 8.

Electron micrograph showing small vascular bundle and portions of contiguous tissues in leaf blade of *Themeda* (Hogsback). In this plane of section, the vascular bundle is surrounded by six chlorenchymatous bundle-sheath cells (BS). The walls of the bundle-sheath cells have a distinct lamellate appearance. The bundle contains two tracheary elements (TE) and two sieve tubes. The sieve tube bordering the tracheary elements is a thick-walled type (TS), and the one bordering the companion cell (CC) a thin-walled type (SE). MC = mesophyll cell; IS = intercellular space; SL = suberin lamella; VP = vascular parenchyma cell. 6 % glutaraldehyde, 2 % OsO₄, 0.05 M cacodylate buffer. X 6100. Inset: Phase contrast micrograph of similar sized bundle. Unlabelled arrow points to thick-walled sieve tube. X 470.



The chloroplasts of the bundle-sheath cells are commonly centrifugally arranged. They are agranal and commonly contain numerous starch grains (Figs 3, 5, 7 and 8).

3.4. The Mesophyll

As seen in transverse sections, most of the mesophyll cells—the Kranz mesophyll cells—are in direct contact with and radially arranged around the chlorenchymatic bundle sheaths. In this view, the mesophyll cells appear “palisade-like” (e.g. Fig. 4); however, in longitudinal sections (Figs 9–11) they can be seen to be deeply lobed or branched.

During examination of transverse sections, the impression was gained that the laminae of *T. Alice* and *T. Hogsback* contained a greater volume of intercellular space than those of *T. Fish River*. In order to confirm this impression, the percentage volume of intercellular space was determined in samples of fresh lamina material from the three provenances. The results of those experiments substantiate the anatomical observations. TFR contained the lowest percentage air volume (3.78 %) and TH the greatest (6.68 %), with TA (6.06 %) close behind TH. The variance was such that no overlap existed between provenances.

4. DISCUSSION

As is typical of the Poaceae, the longitudinal vascular bundles in the leaf blades of *Themeda triandra* can be divided, for descriptive purposes, into three orders, or groups, according to size and structure (Ellis, 1976). The criteria used to distinguish among orders during the present study are essentially the same as those used in light microscope studies: first order (large) bundles are distinguished by the presence of large metaxylem vessels and protoxylem, and an association with hypodermal sclerenchyma girders; second order (intermediate) bundles by the absence of large metaxylem vessels and protoxylem and an association with hypodermal sclerenchyma strands and/or girders; third order (small) bundles by the absence of large metaxylem vessels and protoxylem, and of hypodermal sclerenchyma. In addition, small bundles often are described as having indistinguishable xylem and phloem elements at the light microscope level (Metcalf, 1960; Ellis, 1976),

FIGS. 9–11.

Longitudinal sections showing portions of small vascular bundles of *Themeda triandra* Fish River (Fig. 9), Alice (Fig. 10), and Hogsback (Fig. 11) provenances. Note the deeply lobed mesophyll cells bordering the bundle sheaths (BS) both ad- and abaxially. P = phloem; TE = tracheary element; unlabelled arrows point to sieve plates or thick-walled sieve tubes bordering xylem. Toluidine blue. All X 680.

It is pertinent to note that the so-called orders or sizes of bundles intergrade with one another, and that many intermediate forms exist. Little is known about the spatial, developmental, or functional relationships of the various sized bundles in grass leaves. This problem merits detailed study.

T. triandra represents the second C_4 grass species known to have both thick- and thin-walled sieve tubes in the phloem of its leaf blade bundles. Both types of sieve tube also occur in the leaf blades of *Zea mays*, but apparently in only the small and intermediate bundles (Evert, Eschrich and Heyser, 1978). In *T. triandra*, all three bundle orders have both sieve-tube types; whether the median bundles also have both types remains to be determined. Thick- and thin-walled sieve tubes have been recorded in the longitudinal bundles of the leaf blades of two C_3 grasses, *Triticum aestivum* (Kuo and O'Brien, 1974) and *Oryza sativa* (Miyake and Maeda, 1976). The significance or role of the thick-walled sieve tubes remains to be determined (Evert, 1980).

The leaf blade anatomy of *T. triandra* is typical of that of other Andropogoneae: vascular bundles surrounded by a single layer of bundle-sheath cells with centrifugally-arranged, agranal chloroplasts (Carolín, Jacobs and Veski, 1973). Moreover, on the basis of its anatomy, *T. triandra* has been classified as a XyMS- C_4 species; that is, a species in which cells are absent between the metaxylem vessels and the laterally adjacent chlorenchymatous bundle sheath in at least some of the primary bundles of the blade (Hattersley and Watson, 1976). Significantly, Hattersley and Watson (1976) found a perfect correlation between NADP-malic enzyme type species and the XyMS character. All were XyMS-.

The chlorenchymatous bundle sheath of panicoid grasses long has been considered to be parenchymatous in nature and homologous to the outer sheath of festucoid grasses (Metcalf, 1960). Recently, Brown (1975) has suggested that, in at least some species of C_4 grasses, the chlorenchymatous bundle sheath, which he refers to as the Kranz sheath, has evolved from a mestome sheath. He now recognises two subtypes of Kranz anatomy in grasses, the M.S. subtype in which the Kranz sheath presumably has evolved from a mestome sheath, and the P.S. subtype in which the Kranz sheath has evolved from a parenchyma sheath (Brown, 1977). Brown (1977) further assumes that all species having M.S. anatomy are NADP-malic enzyme species. *Themeda*, and apparently all of Hattersley and Watson's (1976) XyMS-species, are included among the M.S. subtype. Ellis (1977) has adopted Brown's scheme and, specifically, has assigned *T. triandra* to the Kranz mestome sheath subtype.

During the present study, the descriptive term "chlorenchymatous bundle sheath" was used to refer to the sheaths of the various bundle orders in *T. triandra*. This was done because we feel more ontogenetic and phylogene-

tic evidence is needed before the sheaths in andropogonoid grasses are designated mestome sheaths.

Although the term "Kranz" is used by many present-day workers exclusively in connection with bundle-sheath cells, Haberlandt (1882) originally used the term to describe both the chlorenchymatous bundle sheaths and the mesophyll cells radially arranged around them. Later, Haberlandt (1924) used the term to refer only to the mesophyll cells. In our opinion, the designation Kranz anatomy should include both the Kranz mesophyll and the chlorenchymatous bundle sheath, and Kranz syndrome the collection of anatomical, biochemical, and physiological features related to C_4 photosynthesis.

In their presence of a continuous suberin lamella in outer tangential and radial walls, the bundle-sheath cells in *T. triandra* are similar to those of *Zea mays*, another NADP-malic enzyme species (Evert, Eschrich and Heyser, 1977). Unlike the bundle-sheath cells in *Zea mays*, however, the inner tangential walls of those in *T. triandra* are not suberised at the sites of plasmodesmatal aggregates. Suberin lamellae also occur in the mestome sheath walls of C_3 grasses (O'Brien and Carr, 1970; Carolin, Jacobs and Veski, 1973). In addition to being suberised, the bundle-sheath cell walls in *T. triandra* are lignified, a condition not uncommon in grasses.

Except for the marked difference in percentage volume of intercellular space between the leaf blades of *T. Fish River* and those of *T. Alice* and *T. Hogsback*, no anatomical differences could be discerned among the provenances that might give one a special adaptive advantage over the other. One of the most common characteristics of xeromorphic leaves, however, is a small intercellular-space volume (Esau, 1977). In this respect, *T. Fish River*, with its relatively low volume of intercellular space might be better adapted for growth in the hot, semi-arid Fish River Valley area than the other two provenances.

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